

**MISSOURI DEPARTMENT OF NATURAL RESOURCES
AIR AND LAND PROTECTION DIVISION
ENVIRONMENTAL SERVICES PROGRAM
Standard Operating Procedures**

SOP #: MDNR-WQMS-015B EFFECTIVE DATE: January 9, 2004

SOP TITLE: Sample Collection and Field Handling Procedures for Chlorophyll "a" analysis of
Periphyton Samples

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SUMMARY OF REVISIONS: MDNR-WQMS-015 regarding the collection and handling
of collection chlorophyll samples was divided into parts
(015A and 015B). Part A discusses those samples collected from
surface water, while Part B discusses the collection and handling
procedures of periphyton.

APPLICABILITY: Applicable to all ESP personnel who are involved in the collection
and handling of periphyton samples for chlorophyll "a" analysis.

DISTRIBUTION: MDNR Intranet
ESP SOP Coordinator

RECERTIFICATION RECORD:

Date Reviewed				
Initials				

1.0 SCOPE AND APPLICABILITY

This standard operating procedure will discuss the collection and handling methods of periphyton samples collected from both natural and artificial substrates for chlorophyll “a” analysis.

2.0 BACKGROUND

Unlike phytoplankton (suspended algae), which often does not respond fully to the influences of pollution in rivers for considerable distance downstream, periphyton (attached algae) generally show marked responses immediately below pollution sources. In nutrient (e.g., nitrogen, and phosphate) rich environments, periphyton growth can be abundant, if not excessive. Excessive periphyton growth is not aesthetically pleasing and can have negative impacts on the aquatic resources. The abundance and composition of periphyton at a given location is governed by the water quality, and, therefore, can be used as a tool in assessing the effects of pollutants in lakes and streams.

Chlorophyll “a” analysis of periphyton is an indirect measure of the algal biomass within a given area. Periphyton samples are collected from a known area of substrate either natural (stones, sticks, or macrophytes) or artificial (glass, plastic or tile). Chlorophyll is then extracted and expressed as a concentration of chlorophyll “a” per unit area (e.g., mg/m²).

3.0 HEALTH AND SAFETY

- 3.1 Personnel shall participate in the medical monitoring program in accordance with the Missouri Department of Natural Resources (MDNR) medical monitoring policy. Those personnel routinely exposed to wastewater of domestic origin should be vaccinated for Hepatitis A as described in the MDNR’s Hepatitis A prevention vaccine policy. These policies can be viewed on the MDNR’s intranet Health and Safety information page.
- 3.2 Field personnel who are routinely exposed to wastewater (domestic or animal) are encouraged to protect themselves from water borne illnesses by wearing appropriate personal protective equipment (such as clean disposable gloves and waders) and frequently washing their hands with soap and water.

4.0 PERSONNEL QUALIFICATIONS

Field personnel will have at a minimum attended either the department-sponsored inspection and enforcement training or received training from a MDNR employee knowledgeable of the following standard operating procedures.

- MDNR-FSS-001 *Required/Recommended Containers, Volumes, Preservatives, Holding Times and Special Sampling Considerations*
- MDNR-FSS-002 *Field Sheet and Chain-of-Custody Record*
- MDNR-FSS-003 *Sample Numbering and Labeling*
- MDNR-FSS-005 *General Sampling Considerations Including the Collection of Grab, Composite, and Modified Composite Samples from Streams and Wastewater Flows*
- MDNR-FSS-018 *Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab*

5.0 SURVEY PREPARATION

In general, chlorophyll “a” analysis is performed in conjunction with a wasteload allocation/special stream study. Therefore, the preparation procedures should follow those indicated in the Wasteload Allocation/Special Stream Studies project procedure and any other applicable sampling procedures.

6.0 GENERAL CONSIDERATIONS

- 6.1 Specific sampling strategies will depend on the scope of the study. Below are examples of how chlorophyll “a” analysis may aid in water quality assessments (20th Edition of Standard Methods, section 10010):
 - to aid in the interpretation of chemical analyses.
 - to document short- and long-term variability in water quality caused by natural phenomena and/or human activities.
 - to provide data on the status of an aquatic system on a regular basis.
 - to correlate the biological mass or components with water chemistry or conditions.
- 6.2 When collecting periphyton samples for chlorophyll “a” analysis, it is recommended that upstream and downstream periphyton samples also be collected for identification purposes. For additional information, refer to the 20th Edition of Standard Methods, section 10010.
- 6.3 Because of the “patchy” distribution of periphyton, several samples should be collected and analyzed to obtain a representative chlorophyll estimate.
- 6.4 Collection methods will vary depending on the objectives of the study. For consistency and comparison purposes, periphyton samples should be collected from similar stream conditions. For example, samples should be collected from locations with similar canopy cover, substrate type, flow regimes, depth, and growing seasons.
- 6.5 As discussed in the literature, caution should be used when comparing chlorophyll data collected from different studies, different sites, using different techniques, different substrate types, or at different times of the year.

7.0 SAMPLE SITE SELECTION

Sample site selection will vary depending upon the scope of the study. However, the sample site selection should follow the general guidelines as described in MDNR-FSS-005 “*General Sampling Considerations Including the Collection of Grab, Composite, and Modified Composite Samples from Streams and Wastewater Flows.*” cursory examination of the proposed sites should be conducted to determine stream conditions, accessibility, and the specific needs of the survey. Also, refer to the 20th Edition of Standard Methods, section 10300 B for additional sample site considerations.

8.0 SUPPLY LIST

- periphyton chlorophyll “a” biomass field collection record (attached as Appendix A)
- 100 ft. tag line or measuring tape
- random number generator

- collection tray
- periphyton sampling device (refer to Diagram A.)
- small hard bristled brush (e.g., tooth brush, nail brush)
- straight-edge razor blade or scalpel
- magnesium carbonate (MgCO_3) buffered water
- forceps
- 1.0 μm (nominal) pore size, 47 mm, A/E type, Gelman glass fiber filter
- 110 mm diameter, Whatman filter paper
- membrane filtration apparatus (consisting of filter base and stainless steel or Nalgene funnel)
- Nalgene hand pump with appropriate tubing
- 1 liter vacuum filter flask
- pencil
- paper clips
- sample labels
- jar of desiccant
- cooler with ice
- protective gloves

9.0 SAMPLING AND HANDLING CONSIDERATIONS

- 9.1 Skin secretions (oils and perspiration) break down chlorophyll “a” and, therefore, rubber protective gloves should be worn when handling all samples to be submitted for chlorophyll “a” analysis.
- 9.2 Because chlorophyll is easily broken down in the presence of light, the periphyton samples should be filtered in subdued light (if possible) and shall be stored within a dark container containing desiccant.

10.0 NATURAL SUBSTRATES

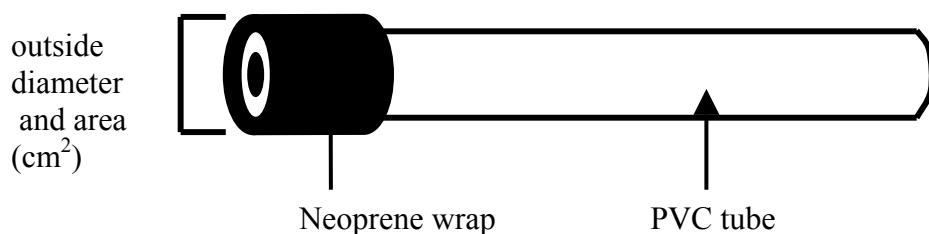
10.1 General Considerations When Using Natural Substrates

- 10.1.1 In water bodies where periphyton growth is excessive and/or long strands of filamentous algae are present, chlorophyll “a” analysis may greatly underestimate the actual biomass value. When this condition occurs, a determination of biomass using the gravimetric method should be considered. Refer to the 20th Edition of Standard Methods, section 10200 I. for additional information regarding determination of biomass using the gravimetric method.
- 10.1.2 Depending upon the scope of the study, periphyton samples may be collected from one or several substrate types (e.g., rocks, sticks, macrophytes, etc.). Many devices have been developed to collect quantitative samples from irregular surfaces. Appropriate techniques for the removal of periphyton from both living and non-living surfaces have been described in literature. This section will describe a collection procedure developed and used by the WQMS.

10.2 Periphyton Sampling Device for Sampling Natural Substrates

Various types of periphyton sampling devices have been described in literature. When sampling from natural substrates, a known area of periphyton is generally scraped from the substrate surface with the aid of a sampling device. The WQMS personnel constructed a basic periphyton sampling device using a PVC tube (with an approximate 1 ½" outside diameter). To act as a water seal and to better mold to uneven surfaces, a strip of Neoprene was wrapped and bonded to the outside edge of PVC tube. The Neoprene was allowed to extend beyond the end of the PVC tube approximately a quarter inch. The sample area was then determined by measuring the outside diameter (cm²) and calculating the area of the sampling device (see Diagram A. below).

Diagram A. Basic Periphyton Sampling Device with Neoprene Wrap



10.3 Collection of Periphyton from Natural Substrate

- 10.3.1 Place the numbered tag line across the width of the stream and divide the stream width into at least three sections.
- 10.3.2 Small cobblestones between 2-4 inches in diameter should be randomly chosen from within each section. The WQMS method was to randomly pick numbers between 1-10. Each number represents the distance from the stream bank or the distance from the point from where the previous cobblestone was collected. The first random number that falls within the designated section should be used. To reduce the effects of water level fluctuations and extreme water flow variances, do not collect stones located close to the stream bank.
- 10.3.3 To avoid periphyton loss when removing the stone from the stream, place and hold the periphyton sampling device on the stones surface prior to lifting it from the streambed. While leaving the periphyton sampler in place, carefully remove the stone from the streambed and carry it to the collection tray.
- 10.3.4 Continue to hold the periphyton sampler in place, and carefully brush and/or scrape all visible algae from the stone surface. Rinse the stone thoroughly with stream water or buffered water prior to removing the periphyton sampler.
- 10.3.5 Remove the periphyton sampler. The area of algae contained underneath the periphyton sampler will be that portion of algae that will be analyzed for chlorophyll. Proceed to section 12.0 for the on-site filtering and handling process of periphyton samples.

- 10.3.6 Thoroughly rinse the periphyton sampler with stream water after each sample collection.

11.0 ARTIFICIAL SUBSTRATES

11.1 General Considerations when using artificial substrates

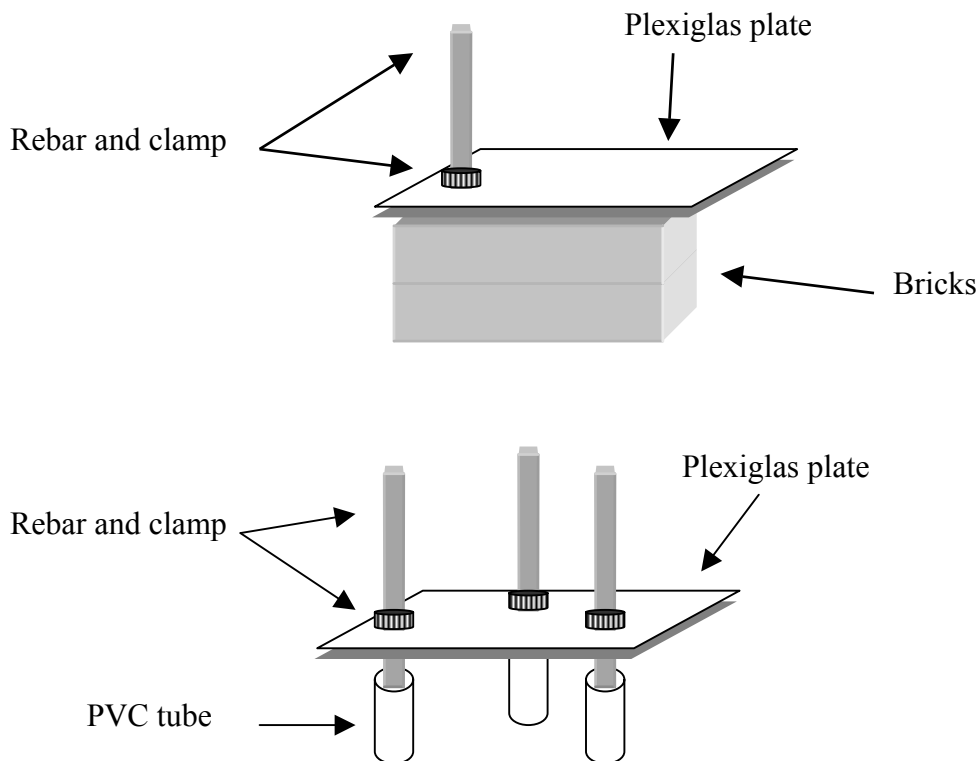
- 11.1.1 It is often difficult to collect quantitative samples from natural substrates with uneven surfaces due to a lack of suitable substrate. To circumvent this problem artificial substrates have been used to provide a uniform surface type, area, and orientation. However, where algal growth is excessive and/or long strands of filamentous algae are present, the chlorophyll “a” analysis may be greatly underestimated because algal mats shade the artificial substrates.
- 11.1.2 For consistency and data comparison within a study period, the artificial substrates should be deployed in the same manner at all site locations (e.g., at the same distance from the water surface or streambed; in the same orientation - horizontal or vertical). In turbid water, literature suggests that artificial substrates should be deployed in a vertical position. If the plates are orientated vertically, all plates should be deployed consistently, either parallel or perpendicular to water flow.
- 11.1.3 In addition, for consistency and comparison purposes, the artificial samplers should be deployed in similar stream conditions (depth, flow, shading, etc.) otherwise, the results may indicate differences in substrate placement and collection rather than the differences in water quality.
- 11.1.4 As discussed previously in section 6.0, due to the “patchy” distribution of periphyton, replicate samples should be collected from each plate and analyzed to obtain a representative chlorophyll estimate.
- 11.1.5 Sufficient colonization time is an important consideration. Literature states proper colonization time will depend on season, water temperature, light, and nutrient availability among other factors. Standard Methods suggests determining the optimum exposure period by testing colonization rates over a period of about six weeks. However, due to time constraints and limited resources this may be impractical. For most circumstances, the colonization period should be at least 14 days.
- 11.1.6 To prevent vandalism, the artificial substrates should be deployed in an inconspicuous area of the stream and away from frequently visited areas.

11.2 Artificial Periphyton Sampling Device

Various types of artificial substrates have been described in literature. The most widely used substrate is the standard, plain glass microscope slide, but other materials such as clear vinyl plastic (e.g., Plexiglas) are also suitable. A basic artificial substrate can be constructed using items such as Plexiglas, rebar, hose clamps, PVC tubes and bricks. See Diagram B for examples of horizontally orientated artificial substrates that have been used

by the WQMS. A numbered grid should be etched or drawn on the underneath side of the plate (i.e., 48 - 2 cm² grids). In addition, to provide a surface that algae can easily attach

Diagram B. Examples of Artificial Substrates Orientated Horizontally



to, the surface of the plate should be lightly sanded.

11.3 Collection of Periphyton from Artificial Substrates

- 11.3.1 2-3 numbered grid squares should be randomly chosen from each plate. With gloved hands and without touching the plexiglas plate, carefully remove the clamp(s) from the rebar. Slowly lift and the plexiglas plate from the streambed and carry it to the collection tray
- 11.3.2 Leaving only the randomly chosen grid squares unscraped, carefully brush and/or scrape all visible algae from the plate surface. Carefully, rinse the outside edge of the plate with stream water or buffered water.
- 11.3.3 Each of the remaining unscraped grids will be analyzed individually for chlorophyll. Proceed to section 12.0 for the filtering and handling process of periphyton samples.

12.0 FILTERING AND HANDLING OF PERIPHYTON SAMPLES

12.1 General Considerations When Filtering and Handling Periphyton Samples.

- 12.1.1 Skin secretions (oils and perspiration) break down chlorophyll “a” and, therefore, rubber protective gloves should be worn when handling all chlorophyll samples.
- 12.1.2 Because chlorophyll is easily broken down in the presence of light, the chlorophyll samples should be filtered in subdued light (if possible) and shall be stored within a dark container containing desiccant.

12.2 Filtering Process

12.2.1 Assemble filtration apparatus:

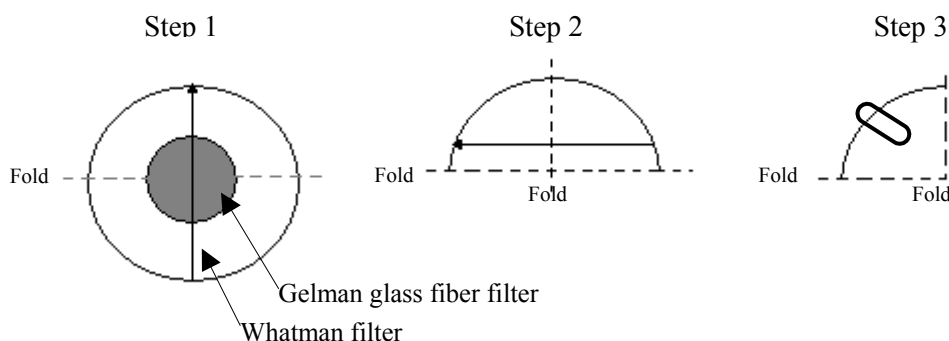
1. Connect the hand pump to the filter flask
2. Place the filter apparatus upon the filter flask
3. Place a Gelman type AE glass fiber on filter apparatus
4. Place funnel on filter apparatus

12.2.2 Assign the filter a sample number (refer to MDNR-FSS-003 *Sample Numbering and Labeling* for additional information). Then using a pencil, carefully label a Whatman filter paper with the sample number and the area (cm²) of periphyton being filtered.

12.2.3 Scrape a known area of periphyton from the substrate for a predetermined period of time (e.g., 30 seconds). Using buffered water thoroughly rinse periphyton from both the substrate surface and scraping device into the filter apparatus.

12.2.4 Using the hand pump, apply a vacuum and filter the excess water until the sample appears “dry”. During filtration process, field personnel should note the amount of vacuum pressure applied. The vacuum pressure should not exceed 10 pounds per square inch (psi) of pressure. Filtering at higher pressures could rupture the algal cells, allowing chlorophyll to pass through the filter and into the filtrate.

12.2.5 Release the vacuum pressure and remove the filter funnel. Using forceps carefully grab the glass fiber filter around the outside edge and place it, chlorophyll side up, on top of the Whatman filter paper. Fold the Whatman filter containing the glass fiber filter in half making sure the glass fiber filter folds completely upon itself. Then proceed to fold the filter in half again so that the information written on the filter paper in step 12.2.2 can be read. See diagram below.



- 12.2.6 Secure the folder filter paper with a paper clip. Place the assigned sample label under the paper clip and place the filtered chlorophyll sample in a dark jar with desiccant.
- 12.2.7 The filtered chlorophyll samples shall be kept in a cooler and on ice. Field personnel should check the cooler periodically to ensure the sample container(s) remain sealed and free of water. Any excess water should be drained from the cooler.
- 12.2.8 Upon return from the field, the samples shall be placed in a freezer and kept frozen until analysis.
- 12.2.9 The maximum holding time for the filtered chlorophyll samples is one (1) month, however, they should be analyzed as soon as possible.

13.0 REFERENCES

Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998

MDNR-FSS-001 Required/Recommended Containers, Volumes, Preservatives, Holding Times and Special Sampling Considerations

MDNR-FSS-002 Field Sheet and Chain-of-Custody Record

MDNR-FSS-003 Sample Numbering and Labeling

MDNR-FSS-005 General Sampling Considerations Including the Collection of Grab, Composite, and Modified Composite Samples from Streams and Wastewater Flows

MDNR-FSS-018 Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab

Appendix A
Periphyton Chlorophyll “a” Biomass Field Collection Record

Periphyton Chlorophyll “a” Biomass Field Collection Record

Stream: _____ County: _____ Sample Collector: _____

Date Collected: _____ Artificial Substrate ☐ or Natural Substrate ☐ (check one)

Sample Number	Site Name	Transect #	Time	Replicate #	Area sampled (cm ²)	Depth to: Plate or Bottom	Comments